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S.O.S. *Pinna nobilis*: A Mass Mortality Event in Western Mediterranean Sea

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A mass mortality event (MME) impacting the bivalve *Pinna nobilis* was detected across a wide geographical area of the Spanish Mediterranean Sea (Western Mediterranean Sea) in early autumn 2016. Underwater visual censuses were conducted across several localities separated by hundreds of kilometers along the Spanish Mediterranean coasts and revealed worrying high mortality rates reaching up to 100% in the center and southernmost coasts of the Iberian Peninsula including Balearic Islands. Populations on the northern coasts of the Spanish Mediterranean Sea seemed to be unaffected (Catalonian region). Histological examination of affected individuals revealed the presence of a haplosporidan-like parasite within the digestive gland being probably the pathogen that causes this mortality. The present MME has spread rapidly, causing high mortality rates in infected populations. Taking into account the degree of impact, the geographic extent, and the high probability that the infection is still in a spreading phase, this might be considered the largest MME ever registered for *P. nobilis* up to date, forcing this emblematic bivalve into a critical viability status over hundreds of kilometers of coast.

Keywords: *Pinna nobilis*, Pinnidae, bivalve, mass mortality events, parasite, Haplosporida, *Pinna rudis*

INTRODUCTION

The Pinnidae family Leach 1819 (order Ostreida, Lemer et al., 2016) is a taxonomic family of large saltwater bivalves commonly known as pen shells or fan mussels. They are filter-feeding molluscs with a long triangular shape with the apex of the shell anchored in the substrate by byssus threads. The shells reach 15–35 cm length, exceptionally up to 120 cm (Schultz and Huber, 2013). This family includes two genera (*Pinna* and *Atrina*, Lemer et al., 2014) with 61 species described worldwide (WoRMS, 2014). Most of the species are distributed in the Indo-Pacific area, with some inhabiting the Caribbean Sea, West Africa, Mediterranean Sea, North East Atlantic, and West America. In the Mediterranean Sea, the endemic bivalve *Pinna nobilis*

Linnaeus 1758 is distributed at depths ranging from 0.5 to 60 m being the largest bivalve of this sea (Zavodnik et al., 1991; Butler et al., 1993). In the twentieth century, *P. nobilis* populations have greatly declined due to anthropogenic activities, including recreational and commercial fishing, ornamental harvesting, and accidental killing by anchoring, bottom nets and trawlers (Vicente and Moreteau, 1991; Richardson et al., 2004; Katsanevakis, 2007; Hendriks et al., 2013; Deudero et al., 2015; Vázquez-Luis et al., 2015). At the present time, this species is legally protected under Annex II of the Barcelona Convention (SPA/BD Protocol 1995), Annex IV of the EU Habitats Directive (EU Habitats Directive 2007), and Spanish Catalog of Threatened Species (Category: Vulnerable, Royal Decree 139/2011). Mass mortality events (hereafter MMEs) represent demographic catastrophes that can affect simultaneously all life stages (Lande, 1993). Recently the magnitudes of MMEs are changing among animal taxa, with increases for birds, marine invertebrates, and fishes (Fey et al., 2015). Analyzing the impact of these events at appropriate scales (spatial and temporal) and biological organization levels (species, populations, communities) is crucial to accurately anticipate future changes in marine ecosystems and propose adapted management and conservation plans (Pairaud et al., 2014). Some MME on bivalve molluscs are consequence of a disease caused by parasites and has been widely studied for commercial bivalve molluscs (Peters, 1988; Elston et al., 1992; Barber, 2004). Among Pinnids, high mortality rates of *Atrina pectinata* (60–90%) and *Atrina lischkeana* (with rates exceeding 60%) have been observed as a consequence of virus-like parasites (Maeno et al., 2006, 2012). Regarding *Pinna nobilis* the existence of one parasite which affects the gonads causing the castration of the animal has been observed (de Gaulejac, 1993). However, studies on diseases or parasites among Pinnids are scarce.

In early autumn 2016 (end September and beginning October 2016) an abnormally high mortality of *P. nobilis* individuals was detected almost simultaneously at several points in the Spanish Mediterranean Sea (south-western Mediterranean Sea) separated by hundreds of kilometers. The main aim of this paper is to provide a comprehensive report on the extent of a *P. nobilis* MME in the SW Mediterranean Sea by providing data on its geographical scale, intensity, depth range, the cause and the timing of the event in the different impacted areas. Finally, we want to raise the alarm to neighboring countries of the Mediterranean Sea.

MATERIALS AND METHODS

Study Areas

This study encompasses a large geographical scale covering the main habitat for *P. nobilis* (*Posidonia oceanica* seagrass meadows) but also other habitats such as *Cymodocea nodosa* seagrass meadows, rocky shores, coastal lagoons, maërl beds, and detritic bottoms. Different research groups involved in the study of the species have conducted censuses to estimate the extent of the event and the mortality rates in the impacted populations at a national level (Spanish Mediterranean Sea). In some cases, affected populations have been studied over the last decade by scientists of various research institutes. Visual censuses were carried out between September 2016 and June 2017 among 137 localities along the Spanish coast (**Figure 1**) and more than 1,600 individuals of pen shells have been registered. Moreover, several diving centers and recreational divers have been contacted (citizen science) in order to obtain widespread information about the current status of the *P. nobilis* population throughout the study area.

Impact Assessment of *P. nobilis* Mortality Event

Data on mortality impact was obtained by both rapid assessments and detailed surveys in monitoring localities (non-destructive surveys). In both types of surveys, number of living, dead and affected (detection of diseased individuals when possible) individuals were recorded to obtain the percentage of the *P. nobilis* population belonging to each state. Additional information such as depth, habitat type, density of individuals, and total area surveyed were also registered when possible. Affected but still living individuals were clearly differentiated, since they present mantle retraction (i.e., not reaching the edge of the valve), a lack of response to stimuli, and slow closing of the valves. Samples of sick and healthy specimens have been collected and histopathological analyses have been carried out in order to identify the agent that could be blamed for the MME. Collection of *P. nobilis* individuals (both sick and healthy specimens) were conducted under permission of "Servei de Protecció d'Espècies" and "Cabrera National Park," both of "Conselleria de Media Ambient, Agricultura i Pesca." A total of 24 individuals from 2 localities separated tens of kilometers were sampled (Andratx and Cabrera National Park (Balearic Islands): 23 and 1 respectively). Samples were fixed in 10% buffered formalin for at least a week, embedded in paraplast, sectioned at 4 μm and routine hematoxilin/eosine stained for light microscope examination.

RESULTS

By the end of September and beginning October 2016 a great mortality of individuals of *Pinna nobilis* was detected across several locations along Spanish Mediterranean Sea (**Figure 1**). The first observations of mortality were reported on the 28th September, 4th and 5th October in the southern and south-east of Iberian Peninsula (Andalusia, Region of Murcia and southern Valencia Community) and Balearic Islands (Formentera and Ibiza). By the third week of October, mortalities close to 90% were detected in almost all the populations under study in the above mentioned localities. In Mallorca the first warnings of anomalous mass mortalities were produced in mid-November 2016. The same pattern was found few months later (March 2017) in populations inhabiting the rest of the Balearic Islands (Menorca and Cabrera) reaching mortalities rates over 90% in the different populations. During March to June 2017, many localities in the above mentioned areas were revisited and the obtained results showed that mortality rates reached in all cases 100%. Regarding Chafarinas Islands located in the Alboran Sea

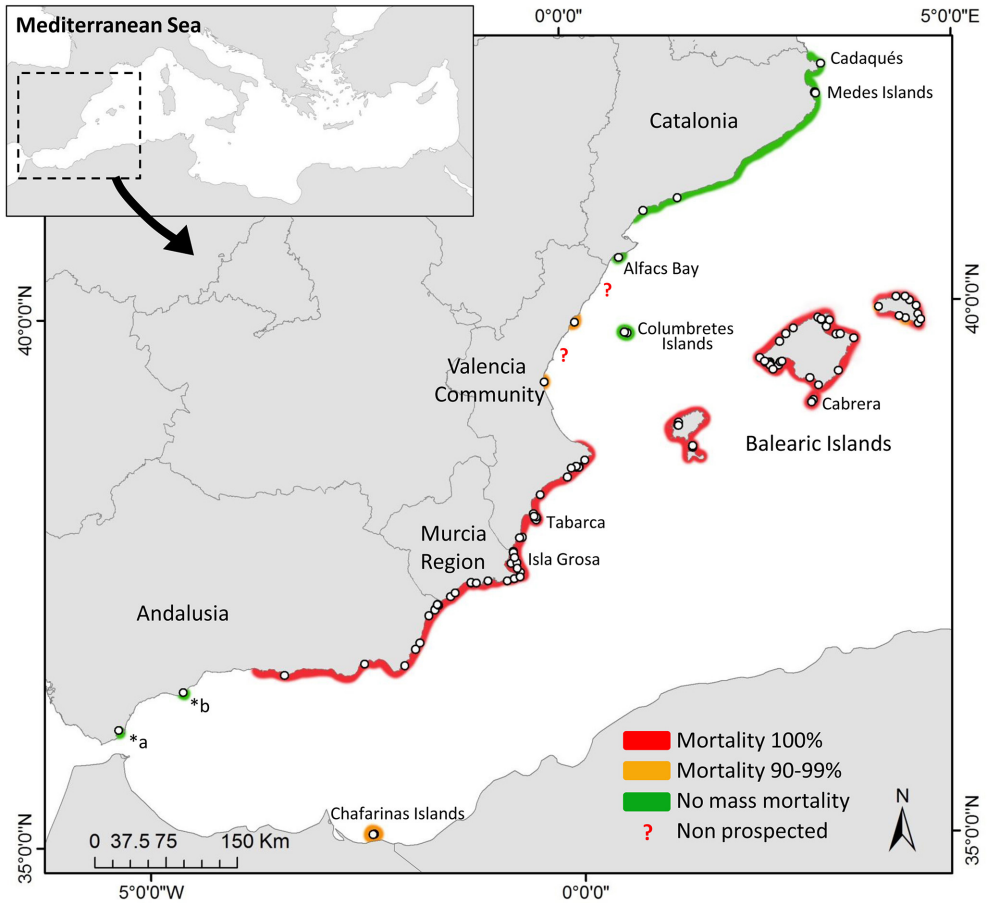

FIGURE 1 | Extent of the mass mortality event on *Pinna nobilis* in western Mediterranean Sea. White dots indicate the sampling localities from September 2016 to June 2017, red shading, mortality of 100% of individuals censed; shaded orange, mortality between 90 and 99% of individuals censed; and green shading, no mass mortality detected. Question marks are places where there are *P. nobilis* populations but it has not been possible to carry out censuses to evaluate the state of the populations in the frame of the MME. *Individuals isolated due to being at their distribution limit: [a]Only four living specimens in Algeciras Bay; and [b]One living specimen.

off the coast of Morocco mortalities of 90% have been detected but this mortality event does not seem to be recent. Affected individuals were found independently of depth, habitat type or size-age (juveniles and adults) of the pen shell individual. In the Columbretes Islands Marine Reserve (located in the north of Valencia Community), populations were unaffected in November 2016. In the same way, data from *P. nobilis* populations inhabiting the northernmost part of the Spanish Mediterranean Sea (Catalonia) obtained in September 2016, showed that these populations were not affected. The most recent data in the area (April-June 2017), corresponding to the populations of the Alfacs Bay in Delta del Ebro (Natural Park), the Medes Islands marine reserve and Cadaqués, indicates that populations in those areas have not been impacted at least for the moment. The congeneric species *P. rudis*, which shares habitat with *P. nobilis* in many of the surveyed areas, seems to be unaffected by the mortality.

Gross signs of sick pen shells, *Pinna nobilis*, were unspecific but include, gaping, slow closing and loss of force of the valves, mantle recession, emaciation and the occurrence of abundant large vesicles full of liquid on the visceral mass. Histological examination revealed the presence of a haplosporidan-like parasite (unknown species, under study) (**Figure 2**) within the digestive gland in the 2 studied localities (in 23 of the 24 individuals analyzed); and in a third locality separated hundreds of kilometers (Darriba, 2017). Numerous plasmodia and sporogonic stages, as well as mature ellipsoidal spores with a lid, were found in the epithelium of digestive tubules. In advanced infected individuals of *P. nobilis*, the epithelium of all the digestive tubules was completely occupied by stages of the parasite; the epithelium height of digestive tubules was reduced, thus resulting in wider lumen. A heavy inflammatory host response was associated with infection, with serious infiltration of the connective tissue of the digestive gland by host hyaline hemocytes. Since food absorption takes place in the digestive gland, a heavy occupation of its epithelium with the parasite should cause severe organ dysfunction, likely leading to host starvation.

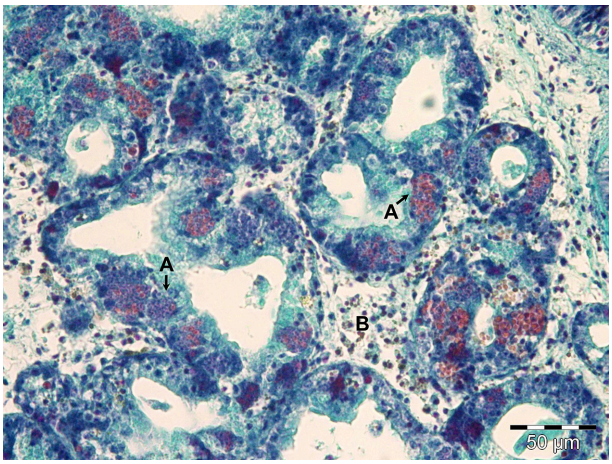

FIGURE 2 | Digestive gland of *P. nobilis*. Mature ellipsoidal uninucleate spores were found in the epithelia of digestive tubules **(A)**. A heavy inflammatory host response, with infiltration of the connective tissue by hyaline hemocytes, was associated with infection **(B)**. Note that the epithelium height of the digestive tubules was reduced, resulting in a wider lumen.

DISCUSSION

The results presented here allow us to infer that a mass mortality event (MME) has affected the populations of *P. nobilis* in a large area of the Spanish Mediterranean Sea (Western Mediterranean Sea) and removing in most cases 100% of pen shell population. Although the proportion of the animal population removed during an MME remains the most widespread approach for defining a MME (Young, 1994; Reed et al., 2003; Garrabou et al., 2009; Ameca et al., 2012), only 9.6% of published MMEs reported information on how MMEs affect population sizes (Fey et al., 2015). This lack of data limits the ability to resolve temporal patterns in the population-level consequences of MMEs. However, reported MMEs frequently remove a substantial proportion of animal populations, including up to 100% of the population (Fey et al., 2015). In our case, given that *P. nobilis* presents the highest population densities in the central Spanish Mediterranean Sea (Southern Catalonia, Valencia Community, Murcia and the Balearic Islands; Guallart and Templado, 2012; Prado et al., 2014) and that in many of those areas the population has suffered a drastic decline close to 100%, it is estimated that the *P. nobilis* population could have been reduced by more than 90% at the national level (Spanish Mediterranean). In the case of the Balearic Islands, the Valencian Community and the Murcia Region some emblematic and long-studied localities for this species were found, such as Tabarca Marine Reserve in Alicante (Garcia-March et al., 2016), the Cabrera National Park in the Balearic Islands (Vázquez-Luis et al., 2014; Deudero et al., 2015) and Isla Grosa Natural Park in Murcia Region (Ruiz et al., 2016) with no individual alive to date. Taking into account that many of the most relevant populations at national level have been impacted by the MME, the consequences in the distribution, potential recovery, and therefore in the viability of the species are unknown and alarming, since most of the populations were already fragmented (Guallart and Templado, 2012). This degree of regression of the Spanish population of 90% should allow a change in the cataloging of the species at the national level from "Vulnerable" to "Endangered" or "Critically Endangered."

The haplosporidan-like parasite detected in moribund individuals is highly probably causing this MME, however samples are still being analyzed and there may be other related causes. Regarding the possible origin of the parasite as it has been pointed out by Darriba (2017), it is impossible to asseverate if this parasite is new infecting *P. nobilis* or if is an old symbiont that has changed leading to a mortality outbreak. Other haplosporidan species, such as *Haplosporidium nelsoni* and *Bonamia* spp., have been responsible for bivalve mass mortalities world-wide (Arzul and Carnegie, 2015). Considering the vast geographic spreading, parasite eradication does not seem feasible because historical attempts to eradicate bivalve mollusk parasites from marine open areas have failed; intermediate or reservoir hosts and parasite long-term resistant stages could contribute to parasite staying even if the specimens of the host species are thoroughly removed from affected areas (Grizel et al., 1986; van Banning, 1991). Therapeutic measures in the open marine environment should also be discarded. Therefore, only prevention measures can be applied. Caution must be taken avoiding the movement of specimens of the species between zones (zoning) and minimizing the risks of infection by transporting parasite infective stages (vessels, equipment, etc.). In the short-term, the possibility of the existence of resistant specimens must be explored; resistance to this infection could be crucial to develop restoration programs using resistant strains produced through selective breeding (Dégremont et al., 2015). It is as well of high importance to search for potentially survived juveniles in the impacted areas as the present MME occurred after spawning of *P. nobilis*, which was observed from May to July in Balearic Islands (Deudero et al., 2017). Additionally, neighboring non-affected populations should be periodically monitored to detect a further spread of the parasite infection.

This unprecedented *P. nobilis* MME has spread very rapidly and has caused high percentages of mortality in infected populations. Taking the degree of impact into account, the geographic extent, and the fact that the event is ongoing, this might be considered the largest MME ever registered for *P. nobilis*, but also among Pinnids worldwide (Maeno et al., 2006, 2012). The possibility that environmental factors mediate in the speed and direction of propagation should be taken into account, since the effect is concentrated only in the South, Southeast and Balearics (e.g., currents) at the moment. Nonetheless, the parasite has progressed over months, therefore can continue spreading across the Mediterranean basin. *P. nobilis* is characterized by high longevity and slow population dynamics with low population replenishment. This event may produce severe consequences, not only in the viability of the affected populations, but, due to the large geographical area impacted, the consequences could be disastrous for the species at least at a Mediterranean sub-basin level. We suggest that neighboring countries urgently adopt

monitoring plans to detect drastic population reductions and identify resistant individuals, to ensure an acceptable level of genetic variability in possible future restocking programs.

AUTHOR CONTRIBUTIONS

MV, EA, AB, JG, AG, IH, SJ, DK, DM, MP, JR, JS, AV, and SD conducted field work or laboratory analyses and help with writing and revision of the manuscript.

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